The chronic myeloid leukaemia stem cell: stemming the tide of persistence.

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ABSTRACT

Chronic myeloid leukaemia (CML) is caused by the acquisition of the tyrosine kinase BCR-ABL1 in a haemopoietic stem cell (HSC), transforming it into a leukaemic stem cell (LSC) that self-renews, proliferates and differentiates to give rise to a myeloproliferative disease. While tyrosine kinase inhibitors (TKI) that target the kinase activity of BCR-ABL1 have transformed CML from a once fatal disease to a manageable one for the vast majority of patients, only ~10% of those who present in chronic phase (CP) can discontinue TKI treatment and maintain a therapy-free remission. Strong evidence now shows that CML LSC are resistant to the effects of TKIs and they persist in all patients on long-term therapy, where they may promote acquired TKI resistance, drive relapse or disease progression and inevitably represent a bottleneck to cure. Since their discovery in patients almost two decades ago, CML LSC have become a well-recognised exemplar of the cancer stem cell and have been characterised extensively with the aim of developing new curative therapeutic approaches based on LSC eradication. This review summarises our current understanding of many of the pathways and mechanisms that promote the survival of the CP CML LSC and how they can be a source of new gene coding mutations that impact in the clinic. We also review recent pre-clinical approaches that show promise to eradicate the LSC, and future challenges on the path to cure.

CML: THE CLASSIC STEM CELL DISEASE

Chronic myeloid leukaemia (CML) is a classic example of a stem cell cancer and arises when the t9;22 translocation (the Philadelphia chromosome)[1-3] occurs in a haemopoietic stem cell (HSC). This event results in the constitutive expression of the fusion tyrosine kinase BCR-ABL1, transforming the HSC into the CML stem cell (referred to here as the leukaemic stem cell or LSC) which then gives rise to a clonal myeloproliferative disease. Early evidence regarding the HSC origins of CML came from observations that transfusion of peripheral blood cells from CML patients into severely neutropenic recipients resulted in temporary homologous BM engraftment and Ph^+ progeny in the blood[4]. This was later explained by the presence of high numbers of mobilised LSC in the peripheral blood of chronic phase (CP) CML patients[5]. A possible haemopoietic progenitor origin of CML was ruled out when BCR-ABL1 expression in murine haemopoietic progenitors failed to confer self-renewal capabilities to BCR-$ABL1^+$ cells, and these cells failed to induce leukaemia in mice[6]. Until very recently, BCR-ABL1 expression was considered sufficient to cause a CML-like disease in mouse models using retrovirus transduction or transgene insertional mutagenesis to express the oncogene in LSC[7-9]. However, issues with BCR-ABL1 copy number, high oncogene expression and/or secondary mutations arising by retroviral or transgene insertional mutagenesis or genomic instability could theoretically contribute to leukaemogenesis. In a recent knock-in model, a single copy of BCR-ABL1 expressed from the endogenous BCR locus was able to confer enhanced BM engraftment, however this model was unable to induce leukaemia[10].

Whilst the cell of origin of CML is generally accepted to be the HSC, several studies implicate an HSC-precursor cell – the multipotent haemangioblast that gives rise to

both haemopoietic and endothelial cells. The BCR-ABL1 fusion can be detected in endothelial cells obtained from BM and peripheral blood of CML patients at varying frequencies[11,12]. These cells show altered intra-cellular signalling and protein expression that may affect crosstalk between LSC and the bone marrow microenvironment (BMM), alter immune-modulation and LSC exit from quiescence into proliferation[13,14]. Collectively these data suggest that the acquisition of BCR-ABL1 in the haemangioblast may contribute to both malignant haemopoiesis and endotheliopoiesis.

THE NATURAL HISTORY OF CML

CML is a rare stem cell disease with an annual incidence of 1-2 cases per 100,000 individuals peaking in the sixth and seventh decades of life[15]. Data derived from atomic bomb survivors[16] suggest that following a latent period of some 7 years, the natural history of CML is for 85-90% of cases to present in CP, but to progress to accelerated phase (AP) and then to either myeloid or lymphoid blast crisis (BC) over a 5 year time frame[17]. However, the mechanism of disease progression is complex and disease behaviour is highly variable for individual patients, with some progressing within a few months and others remaining in stable CP for up to 20 years. This heterogeneity between patients may relate to the mutations subsequently acquired in the BCR-ABL1 clone[18], variations in gene expression patterns between patients[19], or the subtype of HSC in which BCR-ABL1 is first expressed - with recent evidence delineating multiple HSC subsets defined by variably fixed lineage potentialities, transcriptional profiles and phenotypes[20-23]. Furthermore, intriguing work on pre-leukaemia[24,25] and the detection of BCR-ABL1 in blood cells of normal individuals[26,27] presents the possibility that heterogeneity could also be driven by mutations acquired before or after BCR-ABL1, or other

factors such as deregulation and skewing of lineage specification, clonal haemopoiesis, DNA damage, activation of inflammatory responses, and epigenetic alterations, all of which occur in haemopoiesis during aging[28,29]. Some, or all, of these factors may also be required for, or contribute to, disease development in mouse models of CML.

LSC PERSISTENCE: A BOTTLENECK TO CURE

The introduction of a potent BCR-ABL1 tyrosine kinase inhibitor (TKI), imatinib, almost two decades ago, followed by subsequent generations of TKI (dasatinib, nilotinib, bosutinib, ponatinib) has transformed the management of CML[30,31]. What was once a universally fatal disorder, unless treated with an allogeneic transplant, is now well controlled in the outpatient setting and overall survival has improved significantly (http://seer.cancer.gov/statfacts/html/cmyl.html) with the majority of patients requiring life-long TKI. In keeping with disease heterogeneity, patient responses to TKI are also variable. The majority of cases (50-70%) achieve major molecular response (MMR) where BCR-ABL1 levels detectable by quantitative PCR (qPCR) in the blood show a 3 $\log_{10}$ fold reduction, i.e., 0.1%, compared to a standardised baseline (reviewed elsewhere[32]). However, patient to patient variation in leukaemic cell blood counts at diagnosis, and variations in BCR-ABL1 expression between early and late stages of cell differentiation can often confound these interpretations. Approximately 10-20% of all patients develop even deeper molecular responses triggering dose de-escalation and discontinuation/stopping trials (STIM, TWISTER, DADI), where 50% of patients relapse within 12 months[33,34]. When CP relapse occurs, the doubling time (≈ 9 days) for increasing disease burden mirrors the CML disease at diagnosis[35]. A quarter of CP patients fail TKI therapy[36], and approximately half of these cases can be explained by BCR-

ABL1 kinase domain mutations[32,37] but the reason for failure in the remaining patients is unclear.

Ironically, the earliest evidence of CML LSC[38] pre-dated the introduction of TKI and this was followed by definitive evidence of a deeply, but reversibly, quiescent subpopulation of leukaemic cells in patients with CML[39]. In the subsequent years, the consensus view has emerged that virtually all CP patients on TKI therapy and in MMR are not cured of CML and show signs of residual disease burden due to the presence of LSC in the BM (termed “LSC persistence”). In a typical cohort of 100 CP CML patients who undertake TKI therapy over a 5 year period, almost two-thirds of them will have this “LSC persistence” phenotype (Figure 1). Researchers have consistently detected BCR-ABL1^{+} primitive cells in the BM of TKI-treated patients in MMR which are capable of growth in colony forming cell (CFC) and long-term culture initiation cell (LTC-IC) assays, even in patients in deep molecular response with no detectable BCR-ABL1 transcripts by qPCR[40-43]. The most recent of these studies have shown that although LSC are not always detectable in cases of very deep molecular response, most likely due to technical limitations, some patients with no detectable LSC can subsequently relapse after TKI discontinuation[43]. Others have shown that the LSC that persist in patients in MMR express BCR-ABL1 at lower levels than LSC at the point of diagnosis. Furthermore, murine BM cells engineered to express low levels of BCR-ABL1 levels were far less sensitive to imatinib, while those expressing higher levels were prone to *de novo* mutations[44]. These findings point to LSC persistence as a “low mutator” phenotype, perhaps explaining why the majority of these patients do not develop drug resistance or progress to BC. The eradication of the LSC remains a challenge in the

majority of CML patients, a significant bottleneck to cure, and an area of intensive research.

GENERAL FEATURES OF THE LSC

At time of CP diagnosis, BCR-ABL1$^-$ cells co-exist with BCR-ABL1$^+$ cells and enriched CD34$^+$ populations require dual-fluorescent in situ hybridisation (D-FISH) to determine the proportion of cells that carry the Ph$^+$ (usually >90% BCR-ABL1$^+$). The more primitive LSC fraction can be purified by fluorescence-activated cell sorting (FACS) in a variety of ways, giving rise to overlapping, primitive, quiescent populations (Figure 2). Phenotypically and functionally, we define the CP CML LSC as those primitive stem/progenitor cells that show a higher capacity to engraft in immunocompromised mice than bulk CD34$^+$ cells[45], have stem cell properties (self-renewal), are resistant to apoptosis[46,47], are prone to genomic instability[48,49] and have impaired DNA damage responses[50-52]. Since BCR-ABL1 drives survival and proliferation, it is somewhat of a paradox that CML LSC express BCR-ABL1 but can also be quiescent[39] – a feature which may enable them to become refractory to TKI-induced apoptosis. However, TKI also exert a potent anti-proliferative effect on CML CD34$^+$ cells and LSC to induce quiescence[46,53] and subsequent evidence has shown that TKI exert additional effects to subvert a number of pathways to promote survival (see below).

BCR-ABL1 KINASE INDEPENDENT SURVIVAL

To understand why LSC were refractory to the effects of TKI, we exposed CML CD34$^+$ cells to high concentrations of dasatinib for 12 days, and subjected them, in parallel, to BCR-ABL1 knockdown. These *in vitro* studies were complemented *in vivo* using the inducible transgenic SCL-tTA/BCR-ABL model[9]. BCR-ABL1

expression was induced in mice to lead to the development of CML-like disease, then switched off to determine whether the LSC population required BCR-ABL1 for survival, and then induced for a second time to see whether the LSC were still functional and could again drive the development of CML-like disease. In the *in vitro* studies, functional BCR-ABL1$^+$ LSC persisted in culture despite evidence for complete kinase inhibition and significant BCR-ABL1 knock-down. In the mouse model, CML-like disease re-occurred following the second induction of BCR-ABL1. This work demonstrated that LSC survival is not dependent on BCR-ABL1 kinase activity[54] and suggested that BCR-ABL1 may have non-kinase mediated functions that modulate signalling pathways to promote LSC survival. These conclusions were further supported by others who used imatinib to fully inhibit BCR-ABL1 kinase activity in both LSC and quiescent cells[55]. Taken together, these studies concluded that CML LSC were not "oncogene-addicted" and that targeting of BCR-ABL1 kinase activity alone would not eliminate them. Furthermore, this work has led investigators worldwide to search for LSC selective, BCR-ABL1 kinase independent targets and pathways that might offer potential for improved targeting of LSC in CML. To date, a number of mechanisms, pathways and drug-able targets have been proposed to contribute to the TKI-resistant LSC phenotype (Figures 2-3, Tables 1-2).

PI3K/AKT/FOXO SIGNALLING

BCR-ABL1 has been shown to up-regulate PI3K/AKT signalling and AKT-mediated phosphorylation of FOXO transcription factors results in their cytoplasmic localisation where they are inactive (Figure 2). One important consequence of TKI exposure is inhibition of BCR-ABL1 and down-regulation of PI3K/AKT signalling in the LSC (kinase dependent), leading in turn to re-localisation of FOXO1 and

FOXO3a from the cytoplasm to the nucleus, where they modulate expression of CCND1, ATM, CDKN1C, and BCL6 causing a G1 arrest[56,57] and may fuel an anti-apoptotic phenotype. The transcriptional repressor BCL6, a FOXO3A target, is likely to play an important role in this process by repressing the tumour suppressors p53 and ARF. In this respect, TKI exposure permits FOXO3A-mediated up-regulation of BCL6 resulting in a protective, pro-survival effect. Others have shown that the PI3K signalling axis in LSC is also under the control of TGFβ signalling (kinase independent) and blocking this pathway reversed the effects of FOXO nuclear translocation[58,59]. However, the precise mechanism of how this occurs is not fully understood and may not to be completely cell-autonomous.

HEDGEHOG SIGNALLING

Several studies have implicated the hedgehog pathway in the maintenance (self-renewal) and proliferation of the LSC[60-62], where Smoothened (SMO) is a critical mediator. Hedgehog binding to Patched (PTCH) activates SMO which in turn activates the transcription factor GLI1. This leads to reductions of NUMB expression and increased MDM2-mediated degradation of the p53 protein (Figure 2). This would have the effect of suppressing apoptotic responses and/or cell cycle arrest through repression of p53 targets. SMO deletion or pharmacological inhibition in mouse models of CML blocked this pathway and led to loss of LSC[60,61]. However, TKI treatment alone was unable to block this pathway, suggesting that hedgehog signalling was kinase independent. More recently, similar results were obtained using SMO inhibitors in human CML samples *in vitro* and *in vivo* using xenografts in immunocompromised NOD scid gamma (NSG) mice[62].

CANONICAL AND NON-CANONICAL WNT SIGNALLING

β-catenin is a central mediator of both canonical and non-Wnt signalling and has a dual role in regulating cell-to-cell contact through tight junctions and acting as a transcriptional regulator when translocated to the nucleus (Figure 2). In the absence of Wnt signalling, cytoplasmic β-catenin is ultimately phosphorylated by GSK3β and targeted for degradation by an axin-mediated multimeric complex. Nuclear β-catenin is required for self-renewal and survival of normal HSC[63], and it therefore not surprising that it has also been shown to be a key mediator of LSC survival. Loss of β-catenin in a murine model of CML impaired the development of the disease by inhibiting LSC self-renewal[64], and genetic and pharmacological inhibition of β-catenin activity synergised with TKI to target the loss of LSC[65]. Several alternative Wnt-regulated pathways have been implicated in CML LSC. TKI exposure induced the up-regulation of CD70 ligand-induced CD27 signalling[66,67] resulting in β-catenin nuclear translocation and activation of Wnt target genes, including NOTCH, and c-MYC (kinase dependent). TKI exposure also induces a non-canonical Wnt signalling mediated through NFAT signalling which reduces levels of the pro-survival cytokine IL-4[68] (kinase dependent). Fatty acid metabolism was demonstrated to be important in LSC when arachidonate 5-lipoxygenase, encoded by ALOX5 - was shown to be up-regulated in LSC in a kinase independent manner[69] where it is thought to regulate β-catenin levels. Inhibition of ALOX5, through genetic deletion or by pharmacological inhibition in mouse models, targeted the loss of LSC, implicating this component as an important mediator of LSC survival.

JAK/STAT SIGNALLING

The Janus kinases (JAK) family of intracellular non-receptor kinases play important roles in regulating cytokine-mediated signal transduction via the JAK/STAT pathway

(Figure 2). Activation of the signal transducer and activation of transcription 5 (STAT5) was demonstrated in primary CML and CML cell lines twenty years ago[70] and this involves its phosphorylation and translocation to the nucleus where it regulates transcription. Subsequent evidence has also shown that a single null mutation in the STAT5a isoform can attenuate CML-like disease in mouse models[71] and knockdown can impair Ph^+ myeloid colony formation from CML patient samples[72]. Modulating JAK2 activity in human and mouse cell lines reduces BCR-ABL1 and STAT5 signalling[73], and pharmacological inhibition using ruxolitinib resulted in the loss of LSC both *in vitro* and *in vivo*[74], implicating JAK2 as an upstream mediator of a CML JAK/STAT signalling cascade in LSC. However, BCR-ABL1 has also been implicated in the direct activation of STAT5[75] (kinase dependent), suggesting that JAK2 may not be necessary for CML disease maintenance. Furthermore, the re-activation of the tumour suppressor and serine-threonine phosphatase PP2A, through either knockdown or pharmacological inhibition of its repressor SET, has been shown to inhibit BCR-ABL1 and STAT5 activation in CML BC[76]. The scenario, however, is different in LSC, where BCR-ABL1 exerts kinase independent roles to recruit JAK2 to modulate JAK/STAT signalling[77,78] (see also below). Activation of STAT3 has also been implicated in the JAK/STAT cascade, where it exerts a protective effect on CML cells upon exposure to TKI[79]. Inhibition of STAT3 in combination with TKI induced synthetic lethality to target the loss of LSC[80].

GENOMIC INSTABILITY, DNA DAMAGE AND REPAIR

Whether TKI-induced quiescence contributes to LSC persistence in patients is still an open question. Two possible beneficial consequences of TKI treatment would be to reduce the turnover and expansion of LSC in patients and enhance a “low

mutator" phenotype[44]. However, a more cautionary interpretation of these possible benefits has come from the examination of the mechanisms and pathways that contribute to genomic instability in LSC. BCR-ABL1 kinase activity leads to increased levels of reactive oxygen species (ROS)[48,49,81], including H_2O_2, and these lead to oxidative DNA damage, including point mutations and double stranded breaks (DSB). In this regard, the RAC2 GTPase has been shown to alter the function of the mitochondrial respiratory chain complex (MRC-cIII) to generate ROS and DNA damage in LSC, as evidenced by the accumulation of chromosomal aberrations and clinically-relevant BRC-ABL1 kinase domain mutations[49]. This effect was also observed under hypoxia, the conditions that LSC are exposed to in the BM microenvironment, and during exposure to TKI where RAC2 levels were unaffected, thus demonstrating a kinase independent pathway. Inhibition of RAC2 or disruption of the MRC-cIII reduced the level of genomic instability. Similarly, high ROS levels and associated genomic damage were re-capitulated using the transgenic SCL-tTA/BCR-ABL model[48] where both BCR-ABL1 kinase domain mutations and various base pair additions/deletions in genes linked to progression to BC were identified in LSC in both TKI-naïve and TKI-treated mice. Evidence as to why such DNA damage is tolerated in LSC has also emerged. BCR-ABL1 can inhibit mismatch repair to protect cells from apoptosis[50], and can stimulate single-strand annealing, homologous recombination repair (HRR) and non-homologous end-joining, all of which are error-prone in BCR-ABL1 expressing cells[52,81]. Furthermore, LSC are dependent on the alternative RAD52-RAD51 pathway of HRR to deal with DSB rather than BRCA1/2-RAD51, due to the kinase independent down-regulation of BRCA1[82]. While we are unable to reconcile these data with a "low-mutator" phenotype[44], they point to the LSC as a potent source of clinically

relevant mutations, and also argue that CML is constantly evolving at the molecular level even in CP, countering the clinical view that it is a disease of 3 distinct phases.

THE LSC BONE MARROW MICROENVIRONMENT

While the pathways described above have ostensibly been studied as primarily cell-intrinsic or cell-autonomous, it is likely that some, if not all, of them are regulated through interactions between the CML LSC and the BMM - and a number of these interactions have been identified (Figure 3, Table 2), some of which mediate TKI resistance.

LSC adhesion within the BMM is likely to contribute to homing and lodgement – critical steps in LSC engraftment subsequent to transplantation. CD44, expressed on LSC, are ligands for e-selectins and lack of CD44 reduced homing and engraftment of LSC[83]. Similarly, a critical role for selectins and their ligands in engraftment has also been shown[84] and e-selectins can be blocked pharmacologically to reduce the number of LSC. The lectin GAL-3 mediates resistance to TKI through binding β-galactosides on stromal cells and over-expression activated AKT signalling and increased lodgement of LSC in the BM[85]. β1-integrins mediate adhesion of LSC to BM stromal cells, a process likely to be regulated by interferon α[86,87]. TKI-induced up-regulation of N-cadherin in LSC, and adhesion to mesenchymal stem cells led to increased canonical Wnt signalling and protection of the LSC from apoptosis[88]. The CXCL12 ligand and its receptor CXCR4 has been linked to intracellular LYN signalling in LSC[89], and the CXCL12/CXCR4 axis is regulated through CXCL12 cleavage by CD26[90]. Reduced homing capacity of LSC has also been attributed to alterations of the CXCL12/CXCR4 signalling pathway as a result of increased granulocyte-colony stimulating factor (G-CSF)

levels which conferred a selective growth disadvantage to normal HSC[91]. LSC also exert other molecular and phenotypic effects on HSC through extrinsic IL-6 signalling in the CML BMM[92,93]. Indeed, a variety of ligand-receptor mediated signalling pathways regulate CML LSC in the BMM (Figure 3, Table 2).

It is likely that LSC also avoid eradication by modulation of host immune surveillance in the BMM (reviewed in detail elsewhere[94]). In this respect, cytotoxic T lymphocytes (CTLs) are unable to elicit an appropriate immune response against CML cells through CTL exhaustion - and this is believed to be mediated by the interaction of the PD-1 receptor expressed on CTLs with its inhibitory ligand PD-L1 expressed on CML cells. PD-L1 is expressed on patient derived CML cells[95] and on LSC in mouse models of CML[96]. Blockade of the PD-1/PD-L1 interaction in combination with T-cell immunotherapy was able to trigger the loss of LSC, and prevent development of CML-like disease[96]. Our recent work has demonstrated that cytokine-mediated downregulation of MHC-II expression may be an alternative way that LSC evade immune surveillance – and treatment with ruxolitinib or interferon gamma (IFNγ) can reverse this effect *in vitro* and enhance proliferation of responder $CD4^+CD69^+$ T cells in mixed lymphocyte reactions[97]. These examples represent exciting areas of research that could lead to new immune therapy-based therapeutic approaches.

NEW THERAPIES TO TARGET LSC: RECENT APPROACHES

The many examples summarised above illustrate the scope of potentially drug-able targets that have been identified in CML to eradicate LSC (Tables 1-2). Disappointingly, drugs against these targets have yet to be implemented in the clinic as standard of care. In the past 3-4 years, additional drug-able targets and

pathway have been identified, while others previously identified have been further elaborated in pre-clinical studies (Figure 4). Our analysis of global proteomics and transcriptomics in drug-naïve primary patient material (bulk $CD34^+$ cells and LSC) pointed towards a dependency of CML cells on a p53 and c-MYC regulated network[98]. This provided a rationale to use a combination of MDM2 and BET inhibitors (MDM2i, BETi respectively) to target the synergistic eradication of LSC through up-regulation of the p53 apoptotic pathway and down-regulation of c-MYC by both drugs (Figure 4A). Given that BETi acts generally as a transcriptional repressor, how its effects lead to up-regulation of apoptosis in CML LSC is not fully understood, although this appears to be a common phenomenon of BETi in pre-clinical cancer studies[99]. We have also used global epigenetic and transcriptomic analysis of drug-naïve primary patient material to reveal that mis-regulation of the PRC2 complex (including kinase independent down-regulation of EZH1 in LSC) results in the functional dependency of LSC on EZH2 and its biochemical readout H3K27me3. Using murine models, others have also reported that CML LSC are dependent on EZH2[100]. Combining an EZH2 inhibitor (EZH2i) with TKI was highly effective at eradicating the LSC population[101]. Our data supports a model whereby apoptosis is induced in CML LSC through the up-regulation of EZH2 targets upstream of p53 (such as ARF), which could lead to increased p53 levels, or through up-regulation of p53 target genes directly which are normally repressed by EZH2 activity (Figure 4A).

Two groups have shown that activators of the peroxisome proliferator-activated receptor gamma (PPARγ) have increased anti-leukaemic activities in combination with TKI[102,103]. Quiescence of LSCs is regulated by a pathway involving the receptor PPARγ, STAT5, HIF2α, and CITED2 - a master regulator of blood stem cell

quiescence (Figure 4B). Activators of PPARγ result in transcriptional down-regulation of STAT5, whilst TKI block phosphorylation of STAT5 – the combined effects of both drugs significantly down-regulating this pathway and caused LSC to exit quiescence where they were eradicated by TKI[104]. Recently, EZH2 has been shown to be activated by STAT5 in CML cells[105] suggesting possible cross-talk between the effects of PPARy activators and those of EZH2i.

The TKI-mediated up-regulation of CD70 has been further examined to provide a clear rationale for inhibiting non-canonical Wnt/β-catenin signalling in LSC[67]. Upon exposure to TKI, the microRNA miR-29 is down-regulated – the consequence of which is up-regulation of CD70 through the opposing roles of miR-29 on SP1 and DNMT1a regulation (Figure 4C). Thus, antibody-based blockade of the interaction between CD70 and CD27 resulted in a potent loss of LSC in the presence of TKI[67]. Two other routes for inhibiting β-catenin signalling in LSC have also recently been deduced. In the first, BCR-ABL1 interacts directly with JAK2 in a kinase independent manner to activate a JAK2/β-catenin survival/self-renewal pathway that results in inhibition of PP2A and activation of β-catenin (Figure 4D). Use of PP2A activating drugs (PADs) reversed these effects resulting in GSKβ-dependent degradation of β-catenin and eradication of LSC[77]. In the second, another enzyme in fatty acid metabolism arachidonate 15-lipoxygenase (15-LO encoded by ALOX15) has been implicated in the kinase independent up-regulation of β-catenin, although the exact mechanism is unclear. However, pharmacological inhibition of 15-LO in combination with nilotinib on human LSCs *in vitro* appeared synergistic[106]. In addition, the p-selectin SELP appears to be a key down-stream target of 15-LO, which is normally repressed to promote LSC survival. Further pre-clinical studies

and mechanistic studies are required to provide a clearer rationale for taking 15-LO inhibitors into clinical trials as has been done with zileuton which inhibits 5-LO[69].

FUTURE CHALLENGES

We know little about how TKI-resistant LSC clones evolve in patients in MMR and the degree of intra- and inter- patient heterogeneity that is likely to exist – not only at the DNA level, but also with respect to the many pathways that we have identified by studying diagnostic drug-naïve LSC for many years. This is because (i) the TKI-resistant LSC are extremely rare in the BM of these CML patients, and (ii) the LSC cannot be selectively isolated from the normal HSC that reconstitute normal haemopoiesis in the BM subsequent to TKI therapy. Surrogate *in vivo* analysis has also been problematic since the majority of CML primary samples do not engraft well in commonly used immunodeficient mice strains. These issues most likely underpin the failure of many promising new drugs to deliver results in clinical trials. However, recent advances in tracking individual normal and malignant clones in xenograft models using bar-coding[21,107], the development of humanised xenograft models[108], an explosion of single cells technologies[20,109], and the identification of a number of leukaemia-specific cell surface markers, make the analysis of individual LSC or LSC clones much more accessible. Furthermore, several groups have identified markers that discriminate LSC from HSC (CD26[90], IL-1RAP[110], CD25[111] and CD93[112]) but how these will perform in samples from patients in MMR has yet to be determined. For those of us that are intent on curing CML, this new era of game-changing technologies provides some tantalizing prospects that will enable us to finally stem the tide on drug-resistant LSC.

REFERENCES

1. Nowell PC, Hungerford DA. Chromosome studies in human leukemia. II. Chronic granulocytic leukemia. *J Natl Cancer Inst*. 1961;27:1013-1035.
2. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 1973;243(5405):290-293.
3. Heisterkamp N, Stephenson JR, Groffen J, et al. Localization of the c-ab1 oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. *Nature*. 1983;306(5940):239-242.
4. Levin RH, Whang J, Tjio JH, Carbone PP, Frei E, 3rd, Freireich EJ. Persistent Mitosis of Transfused Homologous Leukocytes in Children Receiving Antileukemic Therapy. *Science*. 1963;142(3597):1305-1311.
5. Petzer AL, Eaves CJ, Lansdorp PM, Ponchio L, Barnett MJ, Eaves AC. Characterization of primitive subpopulations of normal and leukemic cells present in the blood of patients with newly diagnosed as well as established chronic myeloid leukemia. *Blood*. 1996;88(6):2162-2171.
6. Huntly BJ, Shigematsu H, Deguchi K, et al. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. *Cancer Cell*. 2004;6(6):587-596.
7. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science*. 1990;247(4944):824-830.
8. Huettner CS, Koschmieder S, Iwasaki H, et al. Inducible expression of BCR/ABL using human CD34 regulatory elements results in a megakaryocytic myeloproliferative syndrome. *Blood*. 2003;102(9):3363-3370.
9. Koschmieder S, Gottgens B, Zhang P, et al. Inducible chronic phase of myeloid leukemia with expansion of hematopoietic stem cells in a transgenic model of BCR-ABL leukemogenesis. *Blood*. 2005;105(1):324-334.
10. Foley SB, Hildenbrand ZL, Soyombo AA, et al. Expression of BCR/ABL p210 from a knockin allele enhances bone marrow engraftment without inducing neoplasia. *Cell Rep*. 2013;5(1):51-60.
11. Gunsilius E, Duba HC, Petzer AL, et al. Evidence from a leukaemia model for maintenance of vascular endothelium by bone-marrow-derived endothelial cells. *Lancet*. 2000;355(9216):1688-1691.
12. Fang B, Zheng C, Liao L, et al. Identification of human chronic myelogenous leukemia progenitor cells with hemangioblastic characteristics. *Blood*. 2005;105(7):2733-2740.
13. Zhu X, Wang L, Zhang B, Li J, Dou X, Zhao RC. TGF-beta1-induced PI3K/Akt/NF-kappaB/MMP9 signalling pathway is activated in Philadelphia chromosome-positive chronic myeloid leukaemia hemangioblasts. *J Biochem*. 2011;149(4):405-414.
14. Li Q, Wu Y, Fang S, et al. BCR/ABL oncogene-induced PI3K signaling pathway leads to chronic myeloid leukemia pathogenesis by impairing immuno-modulatory function of hemangioblasts. *Cancer Gene Ther*. 2015;22(5):227-237.
15. Rohrbacher M, Hasford J. Epidemiology of chronic myeloid leukaemia (CML). *Best Pract Res Clin Haematol*. 2009;22(3):295-302.
16. Ichimaru M, Tomonaga M, Amenomori T, Matsuo T. Atomic bomb and leukemia. *J Radiat Res*. 1991;32 Suppl 2:14-19.
17. Giralt S, Kantarjian H, Talpaz M. The natural history of chronic myelogenous leukemia in the interferon era. *Semin Hematol*. 1995;32(2):152-158.
18. Calabretta B, Perrotti D. The biology of CML blast crisis. *Blood*. 2004;103(11):4010-4022.
19. Yong AS, Szydlo RM, Goldman JM, Apperley JF, Melo JV. Molecular profiling of CD34+ cells identifies low expression of CD7, along with high expression of proteinase 3 or elastase, as predictors of longer survival in patients with CML. *Blood*. 2006;107(1):205-212.

20. Paul F, Arkin Y, Giladi A, et al. Transcriptional Heterogeneity and Lineage Commitment in Myeloid Progenitors. *Cell*. 2015;163(7):1663-1677.
21. Cheung AM, Nguyen LV, Carles A, et al. Analysis of the clonal growth and differentiation dynamics of primitive barcoded human cord blood cells in NSG mice. *Blood*. 2013;122(18):3129-3137.
22. Grover A, Sanjuan-Pla A, Thongjuea S, et al. Single-cell RNA sequencing reveals molecular and functional platelet bias of aged haematopoietic stem cells. *Nat Commun*. 2016;7:11075.
23. Notta F, Zandi S, Takayama N, et al. Distinct routes of lineage development reshape the human blood hierarchy across ontogeny. *Science*. 2016;351(6269):aab2116.
24. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014;506(7488):328-333.
25. Schmidt M, Rinke J, Schafer V, et al. Molecular-defined clonal evolution in patients with chronic myeloid leukemia independent of the BCR-ABL status. *Leukemia*. 2014;28(12):2292-2299.
26. Biernaux C, Loos M, Sels A, Huez G, Stryckmans P. Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. *Blood*. 1995;86(8):3118-3122.
27. Bose S, Deininger M, Gora-Tybor J, Goldman JM, Melo JV. The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. *Blood*. 1998;92(9):3362-3367.
28. Rossi DJ, Bryder D, Zahn JM, et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc Natl Acad Sci U S A*. 2005;102(26):9194-9199.
29. Beerman I, Bock C, Garrison BS, et al. Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. *Cell Stem Cell*. 2013;12(4):413-425.
30. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med*. 1996;2(5):561-566.
31. O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med*. 2003;348(11):994-1004.
32. O'Hare T, Zabriskie MS, Eiring AM, Deininger MW. Pushing the limits of targeted therapy in chronic myeloid leukaemia. *Nat Rev Cancer*. 2012;12(8):513-526.
33. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol*. 2010;11(11):1029-1035.
34. Ross DM, Branford S, Seymour JF, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. *Blood*. 2013;122(4):515-522.
35. Branford S, Yeung DT, Prime JA, et al. BCR-ABL1 doubling times more reliably assess the dynamics of CML relapse compared with the BCR-ABL1 fold rise: implications for monitoring and management. *Blood*. 2012;119(18):4264-4271.
36. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood*. 2013;122(6):872-884.
37. Soverini S, Hochhaus A, Nicolini FE, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. *Blood*. 2011;118(5):1208-1215.
38. Udomsakdi C, Eaves CJ, Swolin B, Reid DS, Barnett MJ, Eaves AC. Rapid decline of chronic myeloid leukemic cells in long-term culture due to a defect at the leukemic stem cell level. *Proc Natl Acad Sci U S A*. 1992;89(13):6192-6196.

39. Holyoake T, Jiang X, Eaves C, Eaves A. Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. *Blood*. 1999;94(6):2056-2064.
40. Bhatia R, Holtz M, Niu N, et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood*. 2003;101(12):4701-4707.
41. Chu S, McDonald T, Lin A, et al. Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. *Blood*. 2011;118(20):5565-5572.
42. Chomel JC, Bonnet ML, Sorel N, et al. Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease. *Blood*. 2011;118(13):3657-3660.
43. Chomel JC, Bonnet ML, Sorel N, et al. Leukemic stem cell persistence in chronic myeloid leukemia patients in deep molecular response induced by tyrosine kinase inhibitors and the impact of therapy discontinuation. *Oncotarget*. 2016; 7(23):35293-352301.
44. Kumari A, Brendel C, Hochhaus A, Neubauer A, Burchert A. Low BCR-ABL expression levels in hematopoietic precursor cells enable persistence of chronic myeloid leukemia under imatinib. *Blood*. 2012;119(2):530-539.
45. Gerber JM, Qin L, Kowalski J, et al. Characterization of chronic myeloid leukemia stem cells. *Am J Hematol*. 2011;86(1):31-37.
46. Graham SM, Jorgensen HG, Allan E, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood*. 2002;99(1):319-325.
47. Copland M, Hamilton A, Elrick LJ, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood*. 2006;107(11):4532-4539.
48. Bolton-Gillespie E, Schemionek M, Klein HU, et al. Genomic instability may originate from imatinib-refractory chronic myeloid leukemia stem cells. *Blood*. 2013;121(20):4175-4183.
49. Nieborowska-Skorska M, Kopinski PK, Ray R, et al. Rac2-MRC-cIII-generated ROS cause genomic instability in chronic myeloid leukemia stem cells and primitive progenitors. *Blood*. 2012;119(18):4253-4263.
50. Stoklosa T, Poplawski T, Koptyra M, et al. BCR/ABL inhibits mismatch repair to protect from apoptosis and induce point mutations. *Cancer Res*. 2008;68(8):2576-2580.
51. Skorski T. BCR/ABL, DNA damage and DNA repair: implications for new treatment concepts. *Leuk Lymphoma*. 2008;49(4):610-614.
52. Cramer K, Nieborowska-Skorska M, Koptyra M, et al. BCR/ABL and other kinases from chronic myeloproliferative disorders stimulate single-strand annealing, an unfaithful DNA double-strand break repair. *Cancer Res*. 2008;68(17):6884-6888.
53. Jorgensen HG, Allan EK, Jordanides NE, Mountford JC, Holyoake TL. Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells. *Blood*. 2007;109(9):4016-4019.
54. Hamilton A, Helgason GV, Schemionek M, et al. Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival. *Blood*. 2012;119(6):1501-1510.
55. Corbin AS, Agarwal A, Loriaux M, Cortes J, Deininger MW, Druker BJ. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. *J Clin Invest*. 2011;121(1):396-409.
56. Hurtz C, Hatzi K, Cerchietti L, et al. BCL6-mediated repression of p53 is critical for leukemia stem cell survival in chronic myeloid leukemia. *J Exp Med*. 2011;208(11):2163-2174.
57. Pellicano F, Scott MT, Helgason GV, et al. The Antiproliferative Activity of Kinase Inhibitors in Chronic Myeloid Leukemia Cells Is Mediated by FOXO Transcription Factors. *Stem Cells*. 2014;32(9):2324-2337.

58. Naka K, Hoshii T, Muraguchi T, et al. TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature*. 2010;463(7281):676-680.
59. Pellicano F, Holyoake TL. Assembling defenses against therapy-resistant leukemic stem cells: Bcl6 joins the ranks. *J Exp Med*. 2011;208(11):2155-2158.
60. Zhao C, Chen A, Jamieson CH, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature*. 2009;458(7239):776-779.
61. Dierks C, Beigi R, Guo GR, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. *Cancer Cell*. 2008;14(3):238-249.
62. Irvine DA, Zhang B, Kinstrie R, et al. Deregulated hedgehog pathway signaling is inhibited by the smoothened antagonist LDE225 (Sonidegib) in chronic phase chronic myeloid leukaemia. *Sci Rep*. 2016;6:25476.
63. Reya T, Duncan AW, Ailles L, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature*. 2003;423(6938):409-414.
64. Zhao C, Blum J, Chen A, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. *Cancer Cell*. 2007;12(6):528-541.
65. Heidel FH, Bullinger L, Feng Z, et al. Genetic and pharmacologic inhibition of beta-catenin targets imatinib-resistant leukemia stem cells in CML. *Cell Stem Cell*. 2012;10(4):412-424.
66. Schurch C, Riether C, Matter MS, Tzankov A, Ochsenbein AF. CD27 signaling on chronic myelogenous leukemia stem cells activates Wnt target genes and promotes disease progression. *J Clin Invest*. 2012;122(2):624-638.
67. Riether C, Schurch CM, Flury C, et al. Tyrosine kinase inhibitor-induced CD70 expression mediates drug resistance in leukemia stem cells by activating Wnt signaling. *Sci Transl Med*. 2015;7(298):298ra119.
68. Gregory MA, Phang TL, Neviani P, et al. Wnt/Ca2+/NFAT signaling maintains survival of Ph+ leukemia cells upon inhibition of Bcr-Abl. *Cancer Cell*. 2010;18(1):74-87.
69. Chen Y, Hu Y, Zhang H, Peng C, Li S. Loss of the Alox5 gene impairs leukemia stem cells and prevents chronic myeloid leukemia. *Nat Genet*. 2009;41(7):783-792.
70. Ilaria RL, Jr., Van Etten RA. P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. *J Biol Chem*. 1996;271(49):31704-31710.
71. Ye D, Wolff N, Li L, Zhang S, Ilaria RL, Jr. STAT5 signaling is required for the efficient induction and maintenance of CML in mice. *Blood*. 2006;107(12):4917-4925.
72. Scherr M, Chaturvedi A, Battmer K, et al. Enhanced sensitivity to inhibition of SHP2, STAT5, and Gab2 expression in chronic myeloid leukemia (CML). *Blood*. 2006;107(8):3279-3287.
73. Samanta A, Perazzona B, Chakraborty S, et al. Janus kinase 2 regulates Bcr-Abl signaling in chronic myeloid leukemia. *Leukemia*. 2011;25(3):463-472.
74. Gallipoli P, Cook A, Rhodes S, et al. JAK2/STAT5 inhibition by nilotinib with ruxolitinib contributes to the elimination of CML CD34+ cells in vitro and in vivo. *Blood*. 2014;124(9):1492-1501.
75. Hantschel O, Warsch W, Eckelhart E, et al. BCR-ABL uncouples canonical JAK2-STAT5 signaling in chronic myeloid leukemia. *Nat Chem Biol*. 2012;8(3):285-293.
76. Neviani P, Santhanam R, Trotta R, et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. *Cancer Cell*. 2005;8(5):355-368.
77. Neviani P, Harb JG, Oaks JJ, et al. PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. *J Clin Invest*. 2013;123(10):4144-4157.
78. Chen M, Gallipoli P, DeGeer D, et al. Targeting primitive chronic myeloid leukemia cells by effective inhibition of a new AHI-1-BCR-ABL-JAK2 complex. *J Natl Cancer Inst*. 2013;105(6):405-423.
79. Traer E, MacKenzie R, Snead J, et al. Blockade of JAK2-mediated extrinsic survival signals restores sensitivity of CML cells to ABL inhibitors. *Leukemia*. 2012;26(5):1140-1143.

80. Eiring AM, Page BD, Kraft IL, et al. Combined STAT3 and BCR-ABL1 inhibition induces synthetic lethality in therapy-resistant chronic myeloid leukemia. *Leukemia*. 2015;29(3):586-597.
81. Nowicki MO, Falinski R, Koptyra M, et al. BCR/ABL oncogenic kinase promotes unfaithful repair of the reactive oxygen species-dependent DNA double-strand breaks. *Blood*. 2004;104(12):3746-3753.
82. Cramer-Morales K, Nieborowska-Skorska M, Scheibner K, et al. Personalized synthetic lethality induced by targeting RAD52 in leukemias identified by gene mutation and expression profile. *Blood*. 2013;122(7):1293-1304.
83. Krause DS, Lazarides K, von Andrian UH, Van Etten RA. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. *Nat Med*. 2006;12(10):1175-1180.
84. Krause DS, Lazarides K, Lewis JB, von Andrian UH, Van Etten RA. Selectins and their ligands are required for homing and engraftment of BCR-ABL1+ leukemic stem cells in the bone marrow niche. *Blood*. 2014;123(9):1361-1371.
85. Yamamoto-Sugitani M, Kuroda J, Ashihara E, et al. Galectin-3 (Gal-3) induced by leukemia microenvironment promotes drug resistance and bone marrow lodgment in chronic myelogenous leukemia. *Proc Natl Acad Sci U S A*. 2011;108(42):17468-17473.
86. Bhatia R, McCarthy JB, Verfaillie CM. Interferon-alpha restores normal beta 1 integrin-mediated inhibition of hematopoietic progenitor proliferation by the marrow microenvironment in chronic myelogenous leukemia. *Blood*. 1996;87(9):3883-3891.
87. Bhatia R, Wayner EA, McGlave PB, Verfaillie CM. Interferon-alpha restores normal adhesion of chronic myelogenous leukemia hematopoietic progenitors to bone marrow stroma by correcting impaired beta 1 integrin receptor function. *J Clin Invest*. 1994;94(1):384-391.
88. Zhang B, Li M, McDonald T, et al. Microenvironmental protection of CML stem and progenitor cells from tyrosine kinase inhibitors through N-cadherin and Wnt-beta-catenin signaling. *Blood*. 2013;121(10):1824-1838.
89. Tabe Y, Jin L, Iwabuchi K, et al. Role of stromal microenvironment in nonpharmacological resistance of CML to imatinib through Lyn/CXCR4 interactions in lipid rafts. *Leukemia*. 2012;26(5):883-892.
90. Herrmann H, Sadovnik I, Cerny-Reiterer S, et al. Dipeptidylpeptidase IV (CD26) defines leukemic stem cells (LSC) in chronic myeloid leukemia. *Blood*. 2014;123(25):3951-3962.
91. Zhang B, Ho YW, Huang Q, et al. Altered microenvironmental regulation of leukemic and normal stem cells in chronic myelogenous leukemia. *Cancer Cell*. 2012;21(4):577-592.
92. Welner RS, Amabile G, Bararia D, et al. Treatment of chronic myelogenous leukemia by blocking cytokine alterations found in normal stem and progenitor cells. *Cancer Cell*. 2015;27(5):671-681.
93. Reynaud D, Pietras E, Barry-Holson K, et al. IL-6 controls leukemic multipotent progenitor cell fate and contributes to chronic myelogenous leukemia development. *Cancer Cell*. 2011;20(5):661-673.
94. Ilander M, Hekim C, Mustjoki S. Immunology and immunotherapy of chronic myeloid leukemia. *Curr Hematol Malig Rep*. 2014;9(1):17-23.
95. Mumprecht S, Schurch C, Schwaller J, Solenthaler M, Ochsenbein AF. Programmed death 1 signaling on chronic myeloid leukemia-specific T cells results in T-cell exhaustion and disease progression. *Blood*. 2009;114(8):1528-1536.
96. Riether C, Gschwend T, Huguenin AL, Schurch CM, Ochsenbein AF. Blocking programmed cell death 1 in combination with adoptive cytotoxic T-cell transfer eradicates chronic myelogenous leukemia stem cells. *Leukemia*. 2015;29(8):1781-1785.
97. Tarafdar A, Hopcroft LEM, Gallipoli P, et al. CML cells actively evade host immune surveillance through cytokine-mediated downregulation of MHC-II expression. *Blood*. 2016;in press.

98. Abraham SA, Hopcroft LE, Carrick E, et al. Dual targeting of p53 and c-MYC selectively eliminates leukaemic stem cells. *Nature*. 2016;534(7607):341-346.
99. Fu LL, Tian M, Li X, et al. Inhibition of BET bromodomains as a therapeutic strategy for cancer drug discovery. *Oncotarget*. 2015;6(8):5501-5516.
100. Xie H, Peng C, Huang J, et al. Chronic myelogenous leukemia initiating cells require Polycomb group protein EZH2. *Cancer Discov*. 2016.
101. Scott MT, Korfi K, Saffrey P, et al. Epigenetic Reprogramming Sensitizes CML Stem Cells to Combined EZH2 and Tyrosine Kinase Inhibition. *Cancer Discov*. 2016.
102. Prost S, Relouzat F, Spentchian M, et al. Erosion of the chronic myeloid leukaemia stem cell pool by PPARgamma agonists. *Nature*. 2015;525(7569):380-383.
103. Glodkowska-Mrowka E, Manda-Handzlik A, Stelmaszczyk-Emmel A, et al. PPARgamma ligands increase antileukemic activity of second- and third-generation tyrosine kinase inhibitors in chronic myeloid leukemia cells. *Blood Cancer J*. 2016;6:e377.
104. Holyoake T, Vetrie D. Cancer: Repositioned to kill stem cells. *Nature*. 2015;525(7569):328-329.
105. Nishioka C, Ikezoe T, Yang J, Yokoyama A. BCR/ABL increases EZH2 levels which regulates XIAP expression via miRNA-219 in chronic myeloid leukemia cells. *Leuk Res*. 2016;45:24-32.
106. Chen Y, Peng C, Abraham SA, et al. Arachidonate 15-lipoxygenase is required for chronic myeloid leukemia stem cell survival. *J Clin Invest*. 2014;124(9):3847-3862.
107. Nguyen LV, Pellacani D, Lefort S, et al. Barcoding reveals complex clonal dynamics of de novo transformed human mammary cells. *Nature*. 2015;528(7581):267-271.
108. Goyama S, Wunderlich M, Mulloy JC. Xenograft models for normal and malignant stem cells. *Blood*. 2015;125(17):2630-2640.
109. Wilson NK, Kent DG, Buettner F, et al. Combined Single-Cell Functional and Gene Expression Analysis Resolves Heterogeneity within Stem Cell Populations. *Cell Stem Cell*. 2015;16(6):712-724.
110. Jaras M, Johnels P, Hansen N, et al. Isolation and killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-1 receptor accessory protein. *Proc Natl Acad Sci U S A*. 2010;107(37):16280-16285.
111. Sadovnik I, Hoelbl-Kovacic A, Herrmann H, et al. Identification of CD25 as STAT5-Dependent Growth Regulator of Leukemic Stem Cells in Ph+ CML. *Clin Cancer Res*. 2016;22(8):2051-2061.
112. Kinstrie R, Horne GA, Morrison H, et al. CD93 Is a Novel Biomarker of Leukemia Stem Cells in Chronic Myeloid Leukemia. *Blood*. 2015;126:49.
113. Bellodi C, Lidonnici MR, Hamilton A, et al. Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. *J Clin Invest*. 2009;119(5):1109-1123.
114. Goussetis DJ, Gounaris E, Wu EJ, et al. Autophagic degradation of the BCR-ABL oncoprotein and generation of antileukemic responses by arsenic trioxide. *Blood*. 2012;120(17):3555-3562.
115. Karvela M, Baquero P, Kuntz EM, et al. ATG7 regulates energy metabolism, differentiation and survival of Philadelphia-chromosome-positive cells. *Autophagy*. 2016;12(6):936-948.
116. Zhang H, Peng C, Hu Y, et al. The Blk pathway functions as a tumor suppressor in chronic myeloid leukemia stem cells. *Nat Genet*. 2012;44(8):861-871.
117. Copland M, Pellicano F, Richmond L, et al. BMS-214662 potently induces apoptosis of chronic myeloid leukemia stem and progenitor cells and synergizes with tyrosine kinase inhibitors. *Blood*. 2008;111(5):2843-2853.
118. Reddiconto G, Toto C, Palama I, et al. Targeting of GSK3beta promotes imatinib-mediated apoptosis in quiescent CD34+ chronic myeloid leukemia progenitors, preserving normal stem cells. *Blood*. 2012;119(10):2335-2345.

119. Zhang B, Strauss AC, Chu S, et al. Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. *Cancer Cell*. 2010;17(5):427-442.
120. Ito K, Bernardi R, Morotti A, et al. PML targeting eradicates quiescent leukaemia-initiating cells. *Nature*. 2008;453(7198):1072-1078.
121. Li L, Wang L, Li L, et al. Activation of p53 by SIRT1 inhibition enhances elimination of CML leukemia stem cells in combination with imatinib. *Cancer Cell*. 2012;21(2):266-281.
122. Naka K, Ishihara K, Jomen Y, et al. Novel oral transforming growth factor-beta signaling inhibitor EW-7197 eradicates CML-initiating cells. *Cancer Sci*. 2016;107(2):140-148.
123. Jin L, Tabe Y, Konoplev S, et al. CXCR4 up-regulation by imatinib induces chronic myelogenous leukemia (CML) cell migration to bone marrow stroma and promotes survival of quiescent CML cells. *Mol Cancer Ther*. 2008;7(1):48-58.
124. Weisberg E, Azab AK, Manley PW, et al. Inhibition of CXCR4 in CML cells disrupts their interaction with the bone marrow microenvironment and sensitizes them to nilotinib. *Leukemia*. 2012;26(5):985-990.
125. Dillmann F, Veldwijk MR, Laufs S, et al. Plerixafor inhibits chemotaxis toward SDF-1 and CXCR4-mediated stroma contact in a dose-dependent manner resulting in increased susceptibility of BCR-ABL+ cell to Imatinib and Nilotinib. *Leuk Lymphoma*. 2009;50(10):1676-1686.
126. Vianello F, Villanova F, Tisato V, et al. Bone marrow mesenchymal stromal cells non-selectively protect chronic myeloid leukemia cells from imatinib-induced apoptosis via the CXCR4/CXCL12 axis. *Haematologica*. 2010;95(7):1081-1089.
127. Laperrousaz B, Jeanpierre S, Sagorny K, et al. Primitive CML cell expansion relies on abnormal levels of BMPs provided by the niche and on BMPRIb overexpression. *Blood*. 2013;122(23):3767-3777.
128. Zhang H, Li H, Xi HS, Li S. HIF1alpha is required for survival maintenance of chronic myeloid leukemia stem cells. *Blood*. 2012;119(11):2595-2607.
129. Ng KP, Manjeri A, Lee KL, et al. Physiologic hypoxia promotes maintenance of CML stem cells despite effective BCR-ABL1 inhibition. *Blood*. 2014;123(21):3316-3326.
130. Preudhomme C, Guilhot J, Nicolini FE, et al. Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. *N Engl J Med*. 2010;363(26):2511-2521.
131. Burchert A, Muller MC, Kostrewa P, et al. Sustained molecular response with interferon alfa maintenance after induction therapy with imatinib plus interferon alfa in patients with chronic myeloid leukemia. *J Clin Oncol*. 2010;28(8):1429-1435.
132. Simonsson B, Gedde-Dahl T, Markevarn B, et al. Combination of pegylated IFN-alpha2b with imatinib increases molecular response rates in patients with low- or intermediate-risk chronic myeloid leukemia. *Blood*. 2011;118(12):3228-3235.
133. Nicolini FE, Etienne G, Dubruille V, et al. Nilotinib and peginterferon alfa-2a for newly diagnosed chronic-phase chronic myeloid leukaemia (NiloPeg): a multicentre, non-randomised, open-label phase 2 study. *Lancet Haematol*. 2015;2(1):e37-46.
134. Hjorth-Hansen H, Stentoft J, Richter J, et al. Safety and efficacy of the combination of pegylated interferon-alpha2b and dasatinib in newly diagnosed chronic-phase chronic myeloid leukemia patients. *Leukemia*. 2016;30(9):1853-1860.
135. Agerstam H, Hansen N, von Palffy S, et al. IL1RAP antibodies block IL1-induced expansion of candidate CML stem cells and mediate cell killing in xenograft models. *Blood*. 2016.
136. Zhang B, Chu S, Agarwal P, et al. Inhibition of interleukin-1 signaling enhances elimination of tyrosine kinase inhibitor treated CML stem cells. *Blood*. 2016.
137. Taverna S, Amodeo V, Saieva L, et al. Exosomal shuttling of miR-126 in endothelial cells modulates adhesive and migratory abilities of chronic myelogenous leukemia cells. *Mol Cancer*. 2014;13:169.

138. Bowers M, Zhang B, Ho Y, Agarwal P, Chen CC, Bhatia R. Osteoblast ablation reduces normal long-term hematopoietic stem cell self-renewal but accelerates leukemia development. *Blood*. 2015;125(17):2678-2688.
139. Zhang B, Li L, Ho Y, et al. Heterogeneity of leukemia-initiating capacity of chronic myelogenous leukemia stem cells. *J Clin Invest*. 2016;126(3):975-991.
140. Schmidt T, Kharabi Masouleh B, Loges S, et al. Loss or inhibition of stromal-derived PlGF prolongs survival of mice with imatinib-resistant Bcr-Abl1(+) leukemia. *Cancer Cell*. 2011;19(6):740-753.
141. Aggoune D, Wessenberger E, Magnani JL, Van Etten RA, Krause DS. The vascular niche is involved in regulating leukemic stem cells in murine chronic myelogenous leukemia. 2014.
142. Krause DS, Fulzele K, Catic A, et al. Differential regulation of myeloid leukemias by the bone marrow microenvironment. *Nat Med*. 2013;19(11):1513-1517.
143. Apperley JF. TWIST it but don't spin it. *Blood*. 2013;122(4):470-471.
144. de Lavallade H, Apperley JF, Khorashad JS, et al. Imatinib for newly diagnosed patients with chronic myeloid leukemia: incidence of sustained responses in an intention-to-treat analysis. *J Clin Oncol*. 2008;26(20):3358-3363.
145. Lucas CM, Wang L, Austin GM, et al. A population study of imatinib in chronic myeloid leukaemia demonstrates lower efficacy than in clinical trials. *Leukemia*. 2008;22(10):1963-1966.
146. Gallipoli P, Shepherd P, Irvine D, Drummond M, Holyoake T. Restricted access to second generation tyrosine kinase inhibitors in the UK could result in suboptimal treatment for almost half of chronic myeloid leukaemia patients: results from a West of Scotland and Lothian population study. *Br J Haematol*. 2011;155(1):128-130.
147. Graham SM, Vass JK, Holyoake TL, Graham GJ. Transcriptional analysis of quiescent and proliferating CD34+ human hemopoietic cells from normal and chronic myeloid leukemia sources. *Stem Cells*. 2007;25(12):3111-3120.
148. Schepers K, Pietras EM, Reynaud D, et al. Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. *Cell Stem Cell*. 2013;13(3):285-299.

TABLES

TARGET OR SURVIVAL FACTOR	PATHWAY	EXEMPLAR INHIBITORS/ ACTIVATORS	REFERENCES	CML CLINICAL TRIAL
ALOX15	β-catenin; PI3K/AKT signalling	PD146176	Chen et al. 2014[106]	No
ALOX5	Wnt/β-catenin signalling	zileuton	Chen et al. 2009[69]	Yes
autophagy	autophagy	chloroquine; bafilomycin A1 As_2O_3	Bellodi et al. 2009[113] Goussetis et al. 2012[114] Karvela et al. 2016[115]	Yes

BCL6	PI3K/AKT/FOX3a/ BCL6 signalling	RI-BPI	Hurtz et al. 2011[56]	No
BLK	MYC/PAX5/BLK/ CDKN1B signalling	n.a.	Zhang et al. 2012[116]	No
CCN	Wnt/Ca^{2+}/NFAT signalling	cyclosporin A	Gregory et al. 2010[68]	No
CD25	JAK/STAT signalling	BEZ235	Sadovnik et al. 2015[111]	No
CD70/CD27	Wnt/β-catenin signalling	αCD70 mAb	Riether et al. 2015[67]	No
c-MYC & TP53	apoptosis	CPI-203; RITA/RG7388	Abraham et al. 2016[98]	No
EZH2	histone H3 K27 tri-methylation	GSK 343; GSK126; EPZ-6438 (tazemetostat)	Scott et al. 2016[101] Xie et al. 2016[100]	No
farnesyl transferases	RAS signalling; protein farnesylation	BMS-214662	Copland et al. 2008[117]	Yes
FOX03A	TGFβ/AKT/FOXO3a/ BCL6 signalling	n.a.	Pellicano et al. 2013[57] Naka et al. 2010[58]	No
GSK3β	Wnt/β-catenin signalling	SB216763	Reddiconto et al. 2012[118]	No
HDACs	histone acetylation	LBH589	Zhang et al. 2010[119]	Yes
JAK2	JAK/STAT signalling	ruxolitinib	Gallipoli et al. 2014[74]	Yes
PML	apoptosis; mTOR repression	arsenic trioxide (As_2O_3)	Ito et al. 2008[120]	Yes
PP2A	JAK/STAT/β-catenin signalling	1,9-dideoxy-forskolin; FTY720	Neviani et al. 2005[76] Neviani et al. 2013[77]	No
PPARγ	STAT5/HIF2α/ CITED2	pioglitazone	Prost et al. 2015[102] Glodkowska-Mrowka et al. 2016[103]	Yes
RAD52	DNA repair	RAD52 F79 peptide aptamer	Cramer-Morales et al. 2013[82]	No
SIRT1	deacetylation of p53	tenovin-6 (TV-6)	Li et al. 2012[121]	No
SMO	Hedgehog signalling	cyclopamine; LDE225;	Zhao et al. 2009[60] Dierks et al. 2008[61] Irvine et al. 2016[62]	Yes
STAT3	JAK/STAT signalling	BP-5087	Eiring et al. 2015[80]	No

TGF-β RI, ALK5	TGFβ/AKT/FOXO3a/ BCL6 signalling	Ly364947; EW-7197	Naka et al. 2010[58] Naka et al. 2016[122]	No

Table 1: Candidate therapeutic targets in CP CML LSCs. Column one: key survival factors/drug targets intrinsic to the LSC. Column two: the roles of these factors/targets in cellular pathways (if known or as proposed by investigators). Column three: exemplar compounds that have been identified as having potential therapeutic value for each factor/target or pathway (if known). Column four: references where the factors/targets were described. Column five: whether factors/targets or compounds have been evaluated in clinical trials that we know of.

TARGET OR SURVIVAL FACTOR	PATHWAY	EXEMPLAR INHIBITORS/ ACTIVATORS	REFERENCES	CML CLINICAL TRIAL
β1-integrins	cell adhesion; proliferation	IFNα	Bhatia et al. 1994[87] Bhatia et al. 1996[86]	Yes
CD26	CXCL12/CXCR4 signalling	glyptins	Herrmann et al. 2014[90]	No
CD44	e-selectin ligands; homing, engraftment	n.a.	Krause et al. 2006[83]	No
CXCR4	CXCL12/CXCR4 axis	AMD3465; AMD3100; plerixafor	Jin et al. 2008[123] Weisberg et al. 2012[124] Dillmann et al. 2009[125] Vianello et al. 2010[126]	Yes
BMP2/4	BMP signalling	n.a.	Laperrousaz et al. 2013[127]	No
GAL-3 (Galectin-3)	BM lodgement	n.a.	Yamamoto-Sugitani et al. 2011[85]	No
HIF1α	hypoxia response	n.a.	Zhang et al. 2012[128] Ng et al. 2014[129]	No
IFNα	immune surveillance; cytokine-mediated proliferation	interferon alfa-2a	Preudhomme et al. 2010[130] Burchert et al. 2010[131] Simonsson et al. 2011[132] Nicolini et al. 2015[133] Hjorth-Hansen et al. 2016[134]	Yes
IL-1RAP/IL-1	NFKβ/AKT signalling	IL-1RAP mAb (mAb81.2)	Jaras et al. 2010[110] Ågerstam et al. 2016[135] Zhang et al. 2016[136]	Yes
IL-6	IL-6Rα signalling from the microenvironment	αIL-6 mAb	Welner et al. 2015[92]	No
LYN	CXCR4/CXCL12 signalling in lipid rafts	methyl-beta-cyclodextrin; PP2	Tabe et al. 2012[89]	No
MHC-II/CIITA	JAK/STAT signalling	ruxolitinib, IFNγ	Tarafdar et al.[97]	Yes
miR-126	exosomal transfer	2-O-Me-miR-126	Taverna et al. 2014[137]	No
N-cadherins	N-cadherin/ Wnt/ β-catenin signalling	ICG001	Zhang et al. 2013[88]	No

JAGGED-1	Notch signalling	n.a.	Bowers et al. 2015[138]	No
MPL	JAK/STAT signalling; engraftment	n.a.	Zhang et al. 2016[139]	No
PD-1/PD-L1	immune surveillance	αPD-L1 mAb αPD-1 mAb	Mumprecht et al. 2009[95] Riether et al. 2015[96]	Yes
PIGF	VEGF signalling: BM angiogenesis; proliferation; metabolism	5D11D4	Schmidt et al. 2011[140]	No
selectins	BM homing; engraftment	GMI-1271	Krause et al. 2014[84] Aggoune et al. 2014[141]	No
TGF-β1	osteoblast → LSC TGFβ signalling	parathyroid hormone (PTH)	Krause et al. 2013[142]	No

Table 2: Candidate therapeutic targets implicated in CP CML LSC/BMM interactions. Column one: key survival factors that act through LSC/BMM interactions. Column two: the roles of these survival factors in cellular pathways (if known or as proposed by investigators). Column three: exemplar compounds that have been identified as having potential therapeutic value for each target (if known). Column four: references where the targets were described. Column five: whether targets or compounds have been evaluated in clinical trials.

FIGURE LEGENDS

Figure 1: The CP CML patients' journey through TKI therapy for 5 years. The schematic shows the clinical outcome for a typical 100 CP CML patients with respect to response to imatinib over the course of 5 years, and the decision-tree leading to discontinuing TKI or switching to 2nd or 3rd generation TKI for various reasons. Outcomes were compiled based on data obtained from various sources[31,143-146]. By the end of year 5: twelve (12) (green segment of the pie chart) of these 100 patients will typically be off TKI and in therapy-free remission (TFR), more than a quarter (26) (red segment of the pie chart) will have failed TKI therapy (even after drug switching or through disease progression to AP or BC), and the majority (62) (amber segment of the pie chart) will remain on long-term TKI therapy but have residual disease due to LSC persistence in their BM.

Figure 2: General features and critical pathways that contribute to CP CML LSC being quiescent, refractory to apoptosis and prone to DNA damage. Typically LSC represent 1-5% of the bulk CML $CD34^+$ cells, are enriched by FACS as $CD34^+CD38^-$, and show more variable levels of Ph^+ cells than bulk CML $CD34^+$ cells. Some researchers also include $Lin^-/CD90^+/CD45RA^-$ cells as part of the $CD34^+CD38^-$ LSC definition[112]. Other FACS approaches can also be used to isolate LSC which employ Hoechst, Pyronin Y and carboxyfluorescein succinimidyl ester (CFSE) intracellular staining in combination with CD34 to identify quiescent/undivided cells[39,46,47,147]. $CD34^+CD38^-$ CML cells from patients at diagnosis which retain high levels of CFSE ($CFSE^{max}$) or are $CD34^+$ and both $Hoechst^{lo}$ and Pyronin Y^{lo}, and survive exposure to TKI, are often considered surrogate *in vitro* models for the TKI-resistant cells that are found in patients with LSC persistence. The schematic diagram of the LSC shows key (but not exhaustive) pathways and components and whether the published evidence points to TKI-dependent (blue) or independent (olive green) mechanisms of regulation. Dotted lines denote translocation of components from the cytoplasm (light red) to the nucleus (white). ROS = reactive oxygen species. TK = tyrosine kinase. Activation and repression are denoted according to convention. Specific details of each pathway are described in the text.

Figure 3: LSC survival signalling in the CP CML bone marrow microenvironment (BMM). The schematic diagram of the BMM shows key (but not

exhaustive) pathway components that mediate signalling between the LSC (light red) and other BMM cell types. HSC is shown in blue. OB = osteoblast cells (tan); CTL = cytotoxic T-cell (turquoise). Ligands involved in various signalling pathways are shown as small coloured spheres. IL-1/IL-1RAP regulates NFKβ signalling in LSC, and can be blocked using a monoclonal antibody to IL-1RAP[135]. MPL, the thrombopoietin (TPO) receptor, regulates JAK/STAT signalling and CML patients with high MPL expression on their LSC have reduced sensitivity to BCR-ABL1 kinase inhibition with TKI, but a higher sensitivity to JAK inhibitors[139]. Leukemic progenitor expansion is driven by exposure of LSC, overexpressing BMPR1B, to BMP2 and BMP4[127].The CML BMM is also thought to over-express the NOTCH ligand JAGGED-1 implicating NOTCH signalling in LSC quiescence[138]. LSC stimulate the production of placental growth factor (PIGF) by BM stromal cells which works in a positive feedback loop to increase angiogenesis of the BM and promote CML cell proliferation through FLT1 (VEGFR1) signalling[140]. Stimulation of BM osteoblasts with parathyroid hormone (PTH) resulted in bone remodelling and production of TGFβ1, eradicated LSC by stimulating TGFβ signalling[142] (the opposite effect to other reports of TGFβ signalling in LSC[58,122]). Similarly, others have shown that expansion of the osteoblast layer of the CML BMM can contribute to creating a hostile environment for HSC – and these effects are mediated by TPO, CCL3 and direct cell-cell interactions that alter TGFβ, NOTCH and pro-inflammatory signalling in the remodelled osteoblasts[148]. Other abbreviations are as described in the text. Other features are as described in Figures 2 and 3.

Figure 4: Recent therapeutic approaches to target the eradication of CP CML LSC. A. Dual targeting of c-MYC and TP53 (p53) or combined treatment with TKI and EZH2 inhibitor (EZH2i)[98,100,101]. Both approaches converge on up-regulating p53-mediated apoptosis through different mechanisms. BET and MDM2 inhibitors (BETi and MDM2i respectively) lead to synergistic repression of c-MYC transcription and up-regulation of p53 target genes. A dependency on EZH2 for LSC survival is accompanied by a TKI-independent down-regulation of EZH1. **B.** Inhibition of STAT5 upstream of the HIF2α-CITED2 pathway that governs LSC quiescence. Combining a PPARγ activator (PPARγa) with TKI[102,103] inhibits STAT5 transcription and STAT5 phosphorylation respectively and down-regulates HIF2α-CITED2 leading to LSC exit from quiescence. **C.** Inhibition of non-canonical Wnt/β-catenin signalling mediated by

CD70/CD27. TKI up-regulates the Wnt/β-catenin pathway by inhibiting miR-29 expression thus facilitating both increased CD70 expression and CD70/CD27 receptor/ligand interaction. Treatment with a monoclonal antibody that blocks the CD70/CD27 interaction (αCD70) in a TKI background blocks the pathway[66,67]. **D.** Activation of PP2A to inhibit a novel CML network driven by JAK2-β-catenin signalling. PP2A activating drugs (PADs) disrupt the PP2A-SET interaction thereby allowing PP2A reactivation which inhibits BCR-ABL1 recruitment of JAK2 (TKI-independent) and impairs β-catenin signalling through GSK-3β activation[77]. **E.** Inhibition of ALOX15 to inhibit β-catenin and PI3K/AKT signalling. Knockdown of ALOX15 or treatment with a 15-LO inhibitor (15-LOi) which blocks ALOX15 enzymatic activity reduced LSC survival in association with reduced PI3K/AKT and β-catenin levels. This “kill” phenotype was rescued by loss of p-selectin (SELP), which is thought to negatively regulate LSC self-renewal and survival[106]. TK = tyrosine kinase. Activation and repression are denoted according to convention. Drug treatments are shown in yellow. Further details are described in the text.

Figure 1. Holyoake and Vetrie

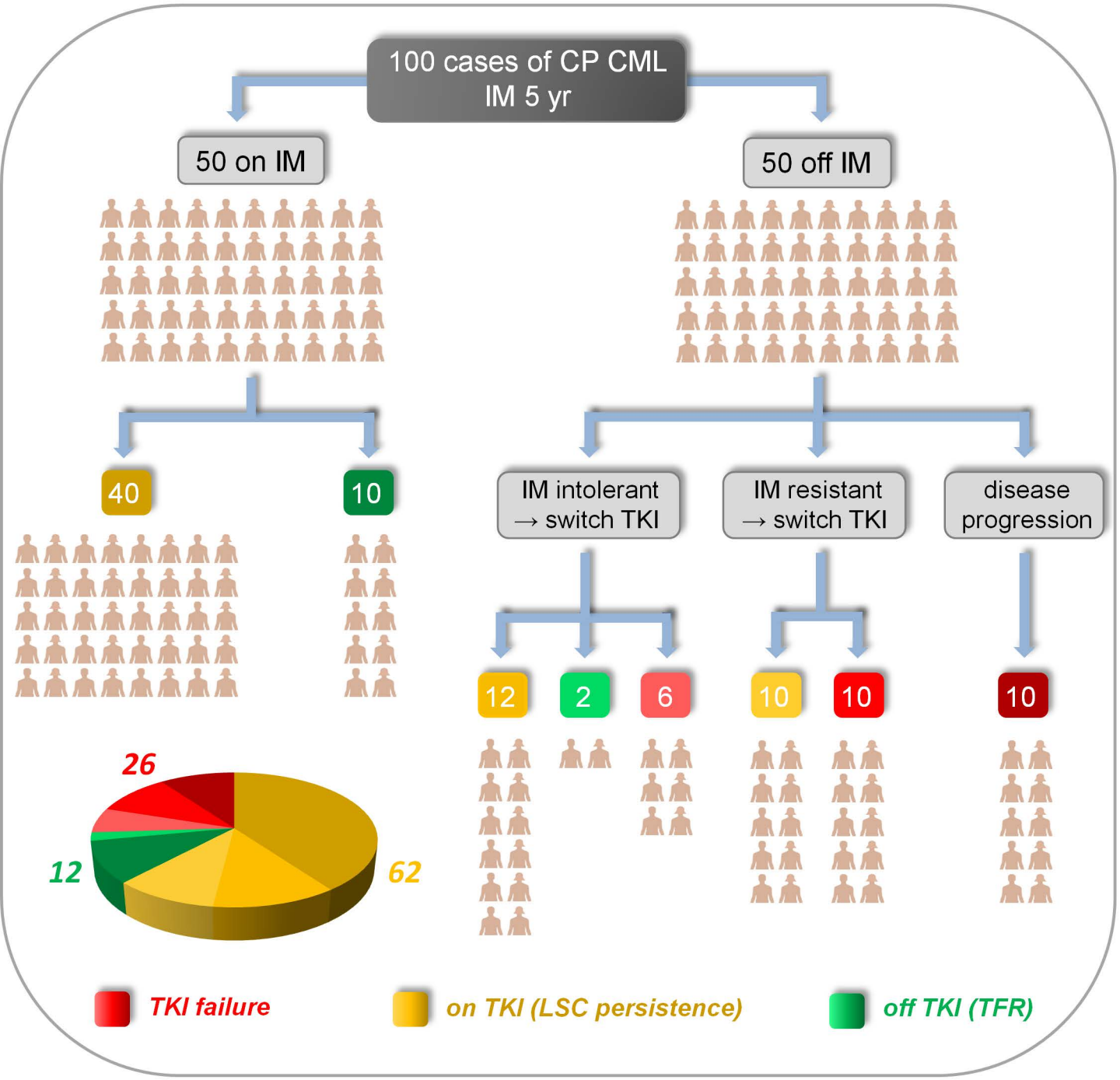


Figure 2. Holyoake and Vetrie

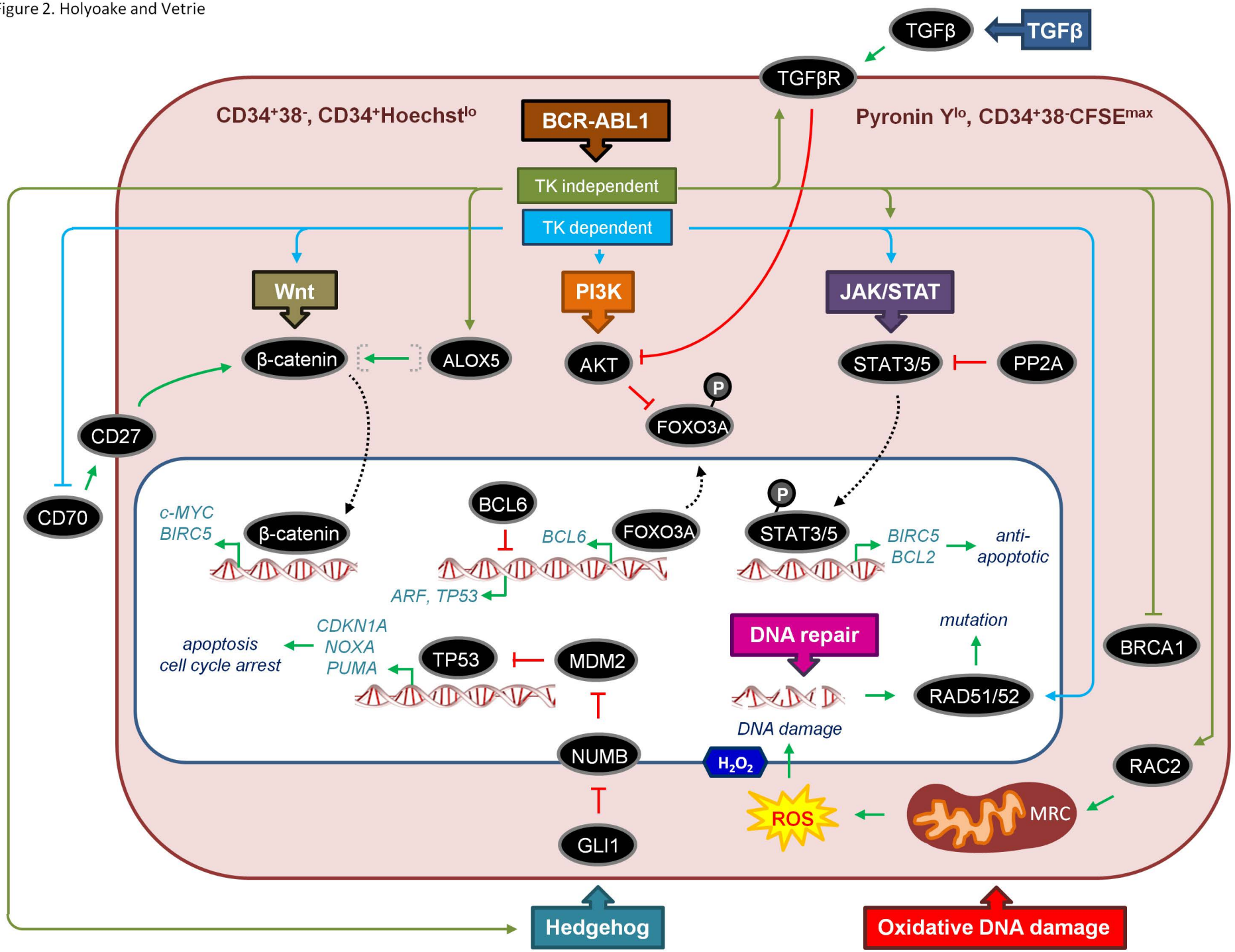
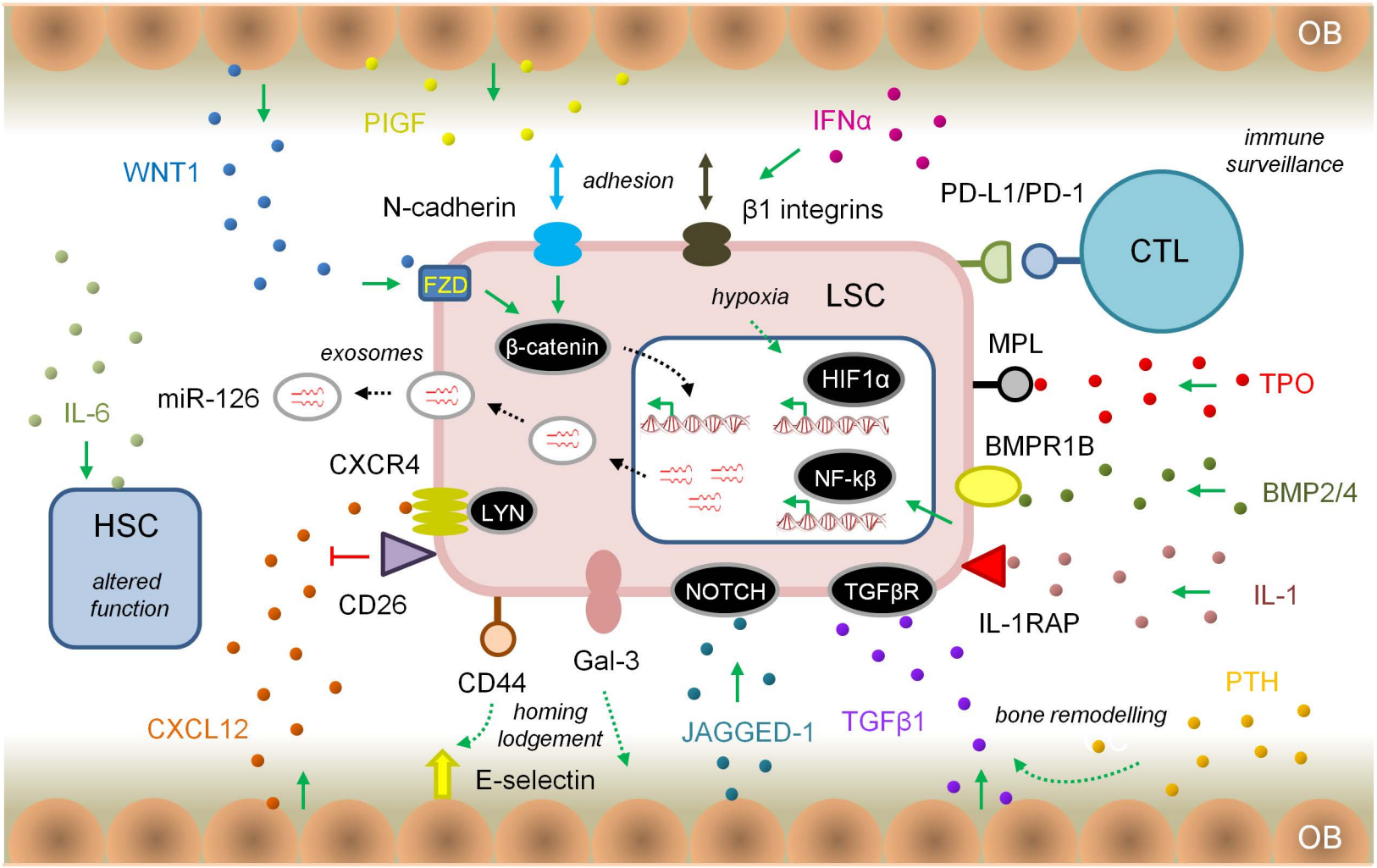


Figure 3. Holyoake and Vetrie



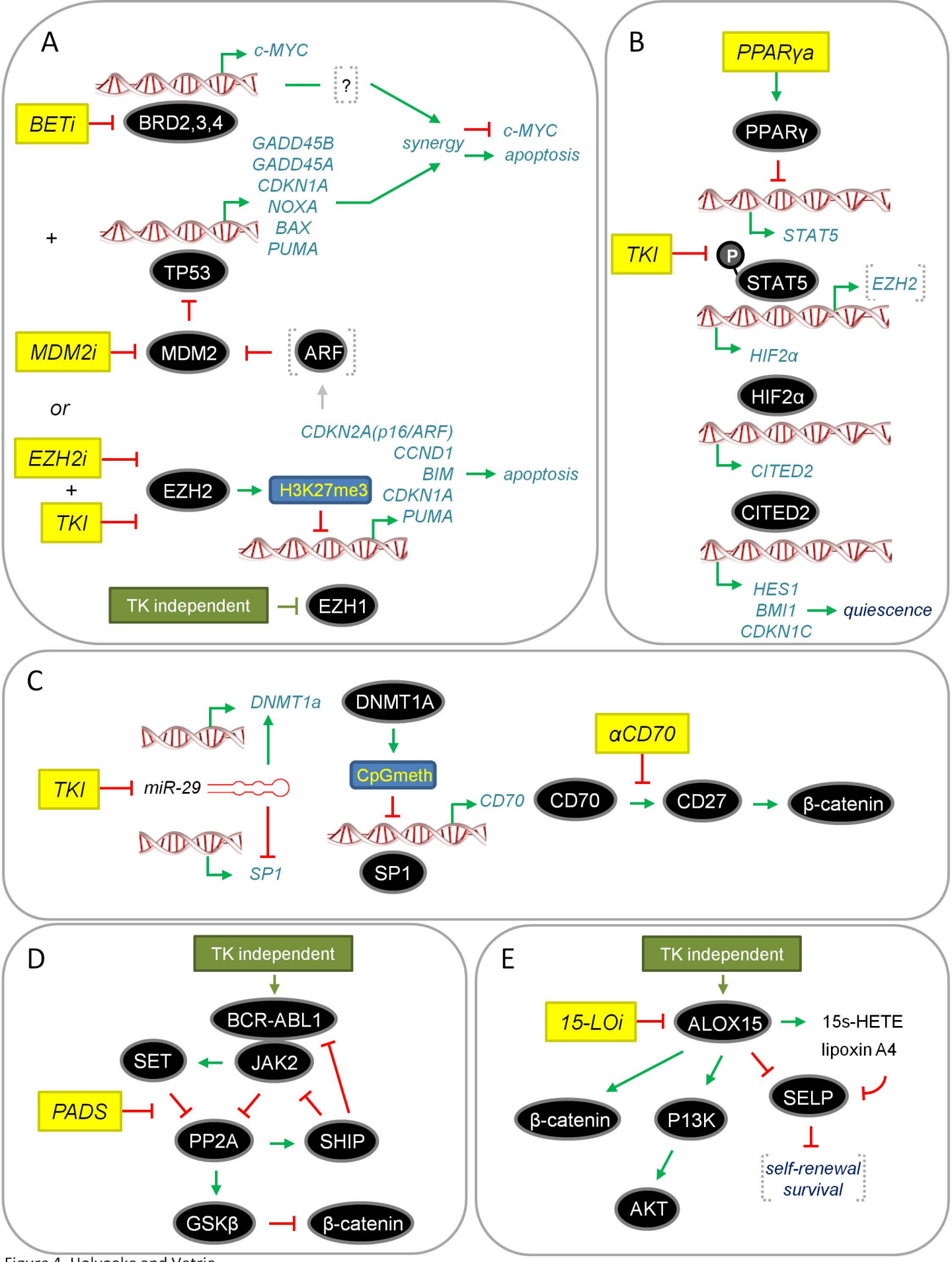


Figure 4. Holyoake and Vetrie